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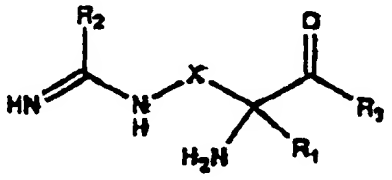
(54) Title: AMIDINO DERIVATIVES USEFUL AS NITRIC OXIDE SYNTHASE INHIBITORS



(I)

(57) Abstract

There is disclosed amidino derivatives of formula (I), pharmaceutical compositions containing these derivatives, and their use in therapy, in particular their use as nitric oxide synthase inhibitors wherein: X is alkyl, alkenyl, alkynyl, $-(CH_2)_kQ(CH_2)l-$ where k is 2 or 3, l is 1 or 2 and Q is 0, Se SiY_2 where Y is alkyl, $S(O)_z$ where z is 0, 1 or 2, NR where R is hydrogen or alkyl, or $-(CH_2)_mA(CH_2)_n-$ where m is 0, 1 or 2, n is 0, 1 or 2, A is 3 to 6 membered aromatic hydrocarbon radical or 3 to 6 membered heterocyclic radical wherein 1 to 3 hetero atoms are oxygen, sulfur or nitrogen and wherein all said radicals may optionally be substituted by one or more substituents such as alkyl, alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino; R_1 may be a bond to X or hydrogen (when Q is Se), hydroxyalkyl, alkyl, alkenyl, alkynyl or haloalkyl and wherein all said radicals may optionally be substituted by one or more substituents such as alkyl, alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino; R_2 is alkyl, alkenyl, alkynyl, aromatic hydrocarbon radical, haloalkyl, hydroxylamine or heterocyclic radical wherein 1 to 3 hetero atoms are oxygen, sulfur or nitrogen and wherein all said radicals may optionally be substituted by one or more substituents such as alkyl, alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino; and R_3 is amino, alkylamine, amino acid, hydroxy, alkoxy, tetrazole or tetrazoloamine.

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AMIDINO DERIVATIVES USEFUL AS NITRIC OXIDE SYNTHASE INHIBITORS

Background of the Invention

Field of the Invention

The present invention relates to novel amidino derivates, pharmaceutical compositions containing these novel compounds, and to their use in therapy, in particular their use as nitric oxide synthase inhibitors.

Related Art

It has been known since the early 1980's that the vascular relaxation brought about by acetycholine is dependent on the presence of the endothelium and this activity was ascribed to a labile humoral factor termed endothelium-derived relaxing factor (EDRF). The activity of nitric oxide (NO) as a vasodilator has been known for well over 100 years and NO is the active component of amylnitrite, glyceryltrinitrite and other nitrovasodilators. The recent identification of EDRF as NO has coincided with the discovery of a biochemical pathway by which NO is synthesized from the amino acid L-arginine by the enzyme NO synthase.

NO is the endogenous stimulator of the soluble guanylate cyclase and is involved in a number of biological actions in addition to endothelium-dependent relaxation including cytotoxicity of phagocytic cells and cell-to-cell communication in the central nervous system (see Moncada et al, Biochemical Pharmacology, 38, 1709-1715 (1989) and Moncada et al, Pharmacological Reviews, 43, 109-142 (1991). It is now thought that excess NO production may be involved in a number of conditions, particularly conditions which involve systemic

hypertension such as toxic shock and therapy with certain cytokines.

The synthesis of NO from L-arginine can be inhibited by the L-arginine analogue, L-N-monomethyl-arginine (L-NMMA) and the therapeutic use of L-NMMA for the treatment of toxic shock and other types of systemic hypertension has been proposed (WO 91/04024 and GB-A-2240041). The therapeutic use of certain other NO synthase inhibitors apart from L-NMMA for the same purpose has also been proposed in WO 91/04024 and in EP-A-0446699.

It has recently become apparent that there are at least two types of NO synthase as follows:

(i) a constitutive, Ca^{++}/calmodulin dependent enzyme that releases NO in response to receptor or physical stimulation.

(ii) a Ca^{++} independent enzyme which is induced after activation of vascular smooth muscle, macrophages, endothelial cells, and a number of other cells by endotoxin and cytokines. Once expressed this inducible NO synthase synthesizes NO for long periods.

The NO released by the constitutive enzyme acts as a transduction mechanism underlying several physiological responses. The NO produced by the inducible enzyme is a cytotoxic molecule for tumor cells and invading microorganisms. It also appears that the adverse effects of excess NO production, in particular pathological vasodilation and tissue damage, may result largely from the effects of NO synthesized by the inducible NO synthase.

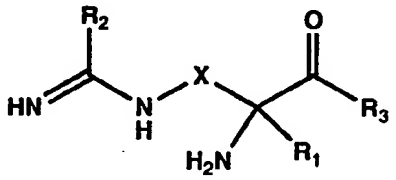
Some of the NO synthase inhibitors proposed for therapeutic use so far, and in particular L-NMMA, are non-selective in that they inhibit both the constitutive and the inducible NO synthase. Use of such a non-

selective NO synthase inhibitor requires that great care be taken in order to avoid the potentially serious consequences of over-inhibition of the constitutive NO-synthase including hypertension and possible thrombosis and tissue damage. In particular, in the case of the therapeutic use of L-NMMA for the treatment of toxic shock it has been recommended that the patient must be subject to continuous blood pressure monitoring throughout the treatment. Thus, while non-selective NO synthase inhibitors have therapeutic utility provided that appropriate precautions are taken, NO synthase inhibitors which are selective in the sense that they inhibit the inducible NO synthase to a considerably greater extent than the constitutive NO synthase would be of even greater therapeutic benefit and easier to use.

WO 93/13055, WO93/24126 and U.S. Patent No. 5,132,453 disclose compounds that inhibit nitric oxide synthesis and preferentially inhibit the inducible isoform of nitric oxide synthase.

Summary of the Invention

The present invention provides amidino derivatives of the formula (I):



(I)

salts, pharmaceutically acceptable esters and prodrugs thereof, wherein

X is lower alkyl, lower alkenyl, lower alkynyl, $-(CH_2)_kQ(CH_2)_l-$ where k is 2 or 3, l is 1 or 2 and Q is O, Se, SiY_2 where Y is lower alkyl, $S(O)_z$ where z is 0, 1 or 2, NR where R is hydrogen or lower alkyl, or $-(CH_2)_mA(CH_2)_n-$ where m is 0, 1 or 2, n is 0, 1 or 2, A is a 3 to 6 membered aromatic hydrocarbon radical or 3 to 6 membered heterocyclic radical wherein 1 to 3 hetro atoms are oxygen, sulfur or nitrogen and wherein all said radicals may optionally be substituted by one or more substituents such as lower alkyl, lower alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino;

R_1 maybe a bond to X, or a radical selected from the group consisting of hydrogen (when Q is Se), hydroxyalkyl, lower alkyl, lower alkenyl, lower alkynyl, or haloalkyl and wherein all said radicals may optionally be substituted by one or more substituents such as lower alkyl, lower alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino;

R_2 is lower alkyl, lower alkenyl, lower alkynyl, aromatic hydrocarbon radical, haloalkyl, hydroxylamine or heterocyclic radical wherein 1 to 3 hetro atoms are oxygen, sulfur or nitrogen and wherein all said radicals may optionally be substituted by one or more substituents such as lower alkyl, lower alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino; and

R_3 is amino, alkylamine, amino acid, hydroxy, lower alkoxy, tetrazole, or tetrazoloamine.

The invention further relates to pharmaceutical compositions comprising a compound of formula (I). Compounds and compositions defined above have usefulness as inhibitors of nitric oxide synthase. These compounds also prefentially inhibit the inducible form over the constitutive form of nitric oxide synthase and in the most prefered form inhibit the inducible form over the constitutive form of nitric oxide synthase by at least 3 fold.

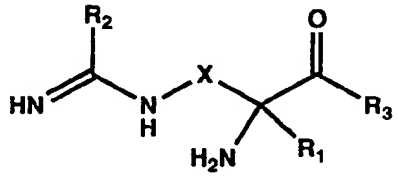
Conditions in which there is an advantage in inhibiting NO production from L-arginine include systemic hypotension associated with septic and/or toxic shock induced by a wide variety of agents; therapy with cytokines such as TNF, IL-1 and IL-2; and as an adjuvant to short term immunosuppression in transplant therapy.

There is also a growing body of evidence that NO may be involved in the degeneration of cartilage which takes place in certain conditions such as arthritis and it is also known that NO synthesis is increased in rheumatoid arthritis. Accordingly, further conditions in which there is an advantage in inhibiting NO production from L-arginine include autoimmune and/or inflammatory conditions affecting the joints, for example arthritis.

Still further conditions in which there is an advantage in inhibiting NO production from L-arginine include inflammatory bowel disease, cardiovascular ischemia, diabetes, cerebral ischemia and other CNS disorders mediated by NO.

Detailed Description of the Invention

A preferred embodiment of the present invention is a compound of the formula (I):



(I)

and salts, and pharmaceutically acceptable ester and prodrugs thereof, wherein:

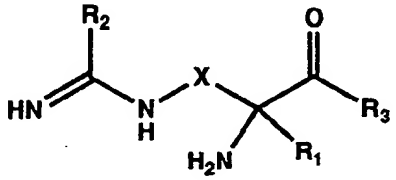
X is lower alkyl, lower alkenyl, lower alkynyl, $-(CH_2)_kQ(CH_2)_l-$ where k is 2 or 3, l is 1 or 2 and Q is O, Se, SiY_2 where Y is lower alkyl, $S(O)_z$ where z is 0, 1 or 2, NR where R is hydrogen or lower alkyl, or $-(CH_2)_mA(CH_2)_n-$ where m is 0, 1 or 2, n is 0, 1 or 2, A is a 3 to 6 membered aromatic hydrocarbon radical or heterocyclic radical wherein 1 to 3 hetro atoms are oxygen, sulfur or nitrogen and wherein all said radicals may optionally be substituted by one or more substituents such as lower alkyl, lower alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino;

R_1 maybe a bond to X, or a radical selected from the group consisting of hydrogen (when Q is Se), hydroxyalkyl, lower alkyl or haloalkyl;

R_2 is lower alkyl, lower alkenyl, lower alkynyl, aromatic hydrocarbon radical , haloalkyl, or hydroxylamine; and

R_3 is amino, alkylamine, natural amino acid, hydroxy, lower alkoxy, tetrazole, or tetrazoloamine.

Another preferred embodiment of the present invention is a compound of the formula (I) :



(I)

and salts, and pharmaceutically acceptable ester and prodrugs thereof, wherein:

X is a lower alkyl of 1 to about 6 carbon atoms, lower alkenyl of 1 to about 6 carbon atoms, lower alkynyl of 1 to about 6 carbon atoms, $-(CH_2)_kQ(CH_2)_l-$ where k is 2 or 3, l is 1 or 2 and Q is O, Se, SiY_2 where Y is lower alkyl, S(O)z where z is 0, 1 or 2, or NR where R is H or lower alkyl; or $-(CH_2)_mA(CH_2)_n-$ where m is 0, 1 or 2, n is 0, 1 or 2, A is a 3 to 6 membered aromatic hydrocarbon radical or heterocyclic radical wherein 1 to 3 hetero atoms are oxygen, sulfur or nitrogen and wherein all said radicals may optionally be substituted by one or more substituents such as lower alkyl, lower alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino;

R_1 is hydrogen (when Q is Se), a hydroxyalkyl group of 1 to about 4 carbon atoms, a lower alkyl group of 1 to

about 4 carbon atoms or a haloalkyl group of 1 to about 4 carbon atoms;

R_2 is lower alkyl of 1 to about 4 carbon atoms, lower alkenyl of 1 to about 4 carbon atoms alkynyl of 1 to about 4 carbon atoms, aromatic hydrocarbon radical of 3 to about 6 carbon atoms and haloalkyl of 1 to about 4 carbon atoms; and

R_3 is an amino, alkylamine of 1 to about 4 carbon atoms, hydroxy, lower alkoxy of 1 to about 4 carbon atoms, tetrazole, tetrazoloamine, or natural amino acid.

Still another preferred embodiment of formula (I) is X is an alkylene group having 3 to 5 carbon atoms and which may optionally be substituted by one or more C_{1-3} alkyl; a group of formula $-(CH_2)_kQ(CH_2)_l-$ where k is 2 or 3, l is 1 or 2 and Q is O, Se, S(O)z where z is 0, 1 or 2; or a group of formula $-(CH_2)_mA(CH_2)_n-$ where m is 0, 1 or 2, n is 0, 1 or 2, A is a 3 to 6 membered carbocyclic or heterocyclic ring;

R1 is hydrogen (when Q is Se), a hydroxyalkyl group of 1 to about 4 carbon atoms, a lower alkyl radical of 1 to about 4 carbon atoms;

R_2 is lower alkyl radical of 1 to about 4 carbon atoms;

R_3 is an amino, alkylamine of 1 to about 4 carbon atoms, hydroxy, lower alkoxy group of 1 to about 4 carbon atoms.

The present invention includes compounds of formula (I) in the form of salts, in particular acid addition salts. Suitable salts include those formed with both organic and inorganic acids. Such acid addition salts

will normally be pharmaceutically acceptable although salts of non-pharmaceutically acceptable salts may be of utility in the preparation and purification of the compound in question. Thus, preferred salts include those formed from hydrochloric, hydrobromic, sulphuric, citric, tartaric, phosphoric, lactic, pyruvic, acetic, succinic, oxalic, fumaric, maleic, oxaloacetic, methanesulphonic, ethanesulphonic, p-toluenesulphonic, benzenesulphonic and isethionic acids. Salts of the compounds of formula (I) can be made by reacting the appropriate compound in the form of the free base with the appropriate acid.

While it may be possible for the compounds of formula (I) to be administered as the raw chemical, it is preferable to present them as a pharmaceutical formulation. According to a further aspect, the present invention provides a pharmaceutical formulation comprising a compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof, together with one or more pharmaceutically acceptable carriers thereof and optionally one or more other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous and intraarticular), rectal and topical (including dermal, buccal, sublingual and intraocular) administration although the most suitable route may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into

association a compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof ("active ingredient") with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the

blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol.

Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges comprising the active ingredient in a flavored basis such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a basis such as gelatin and glycerin or sucrose and acacia.

Preferred unit dosage formulations are those containing an effective dose, as hereinbelow recited, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

The compounds of the invention may be administered orally or via injection at a dose of from 0.1 to 500

mg/kg per day. The dose range for adult humans is generally from 5mg to 2g/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of compound of the invention which is effective at such dosage or as a multiple of the same, for instance, units containing 5mg to 500mg, usually around 10mg to 200mg.

The compounds of formula (I) are preferably administered orally or by injection (intravenous or subcutaneous). The precise amount of compound administered to a patient will be the responsibility of the attendant physician. However, the dose employed will depend on a number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity. Also, the route of administration may vary depending on the condition and its severity.

As utilized herein, the term "lower alkyl", alone or in combination, means an acyclic alkyl radical containing from 1 to about 10, preferably from 1 to about 8 carbon atoms and more preferably 1 to about 6 carbon atoms. Examples of such radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-amyl, hexyl, octyl and the like.

The term "lower alkenyl" refers to an unsaturated acyclic hydrocarbon radical in so much as it contains at least one double bond. Such radicals containing from about 2 to about 10 carbon atoms, preferably from about 2 to about 8 carbon atoms and more preferably 2 to about 6 carbon atoms. Examples of suitable alkenyl radicals include propylenyl, buten-1-yl, isobutenyl, pentenylen-1-yl, 2-2-methylbuten-1-yl, 3-methylbuten-1-yl, hexen-1-yl, hepten-1-yl, and octen-1-yl, and the like.

The term "lower alkynyl" refers to an unsaturated acyclic hydrocarbon radicals in so much as it contains one or more triple bonds, such radicals containing about 2 to about 10 carbon atoms, preferably having from about 2 to about 8 carbon atoms and more preferably having 2 to about 6 carbon atoms. Examples of suitable alkynyl radicals include ethynyl, propynyl, butyn-1-yl, butyn-2-yl, pentyn-1-yl, pentyn-2-yl, 3-methylbutyn-1-yl, hexyn-1-yl, hexyn-2-yl, hexyn-3-yl, 3,3-dimethylbutyn-1-yl radicals and the like.

The term "aromatic hydrocarbon radical" means 4 to about 16 carbon atoms,preferably 6 to about 12 carbon atoms, more preferably 6 to about 10 carbon atoms. Examples of suitable aromatic hydrocarbon radicals include phenyl,naphthyl, and the like.

The term "heterocyclyl radical" means a saturated or unsaturated cyclic hydrocarbon radical with 4 to about 10 carbon atoms, preferably about 5 to about 6; wherein 1 to about 3 carbon atoms are replaced by nitrogen, oxygen or sulfur. The "heterocyclic radical" may be fused to an aromatic hydrocarbon radical. Suitable examples include pyrrolyl, pyridinyl, pyrazolyl, triazolyl, pyrimidinyl, pyridazinyl, oxazolyl, thiazolyl, imidazolyl, indolyl, thiophenyl, furanyl, tetrazolyl, 2-pyrrolinyl, 3-pyrrolinyl, pyrrolindinyl, 1,3-dioxolanyl, 2-imidazonlinyl, imidazolidinyl, 2-pyrazolinyl, pyrazolidinyl, isoxazolyl, isothiazolyl, 1,2,3-oxadiazolyl, 1,2,3-triazolyl, 1,3,4-thiadiazolyl, 2H-pyranyl, 4H-pyranyl, piperidinyl, 1,4-dioxanyl, morpholinyl, 1,4-dithianyl, thiomorpholinyl, pyrazinyl, piperazinyl, 1,3,5-triazinyl, 1,3,5-trithianyl, benzo(b)thiophenyl, benzimidazolyl, quinolinyl, and the like.

The term "lower alkoxy", alone or in combination, means an alkyl ether radical wherein the term alkyl is as defined above and most preferably containing 1 to about 4 carbon atoms. Examples of suitable alkyl ether radicals include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, iso-butoxy, sec-butoxy, tert-butoxy and the like.

The term "halogen" means fluorine, chlorine, bromine or iodine.

The term amino acid means an amino alkanoic wherein the amino can be postioned anywhere on the alkyl group. The alkyl is as is defined above.

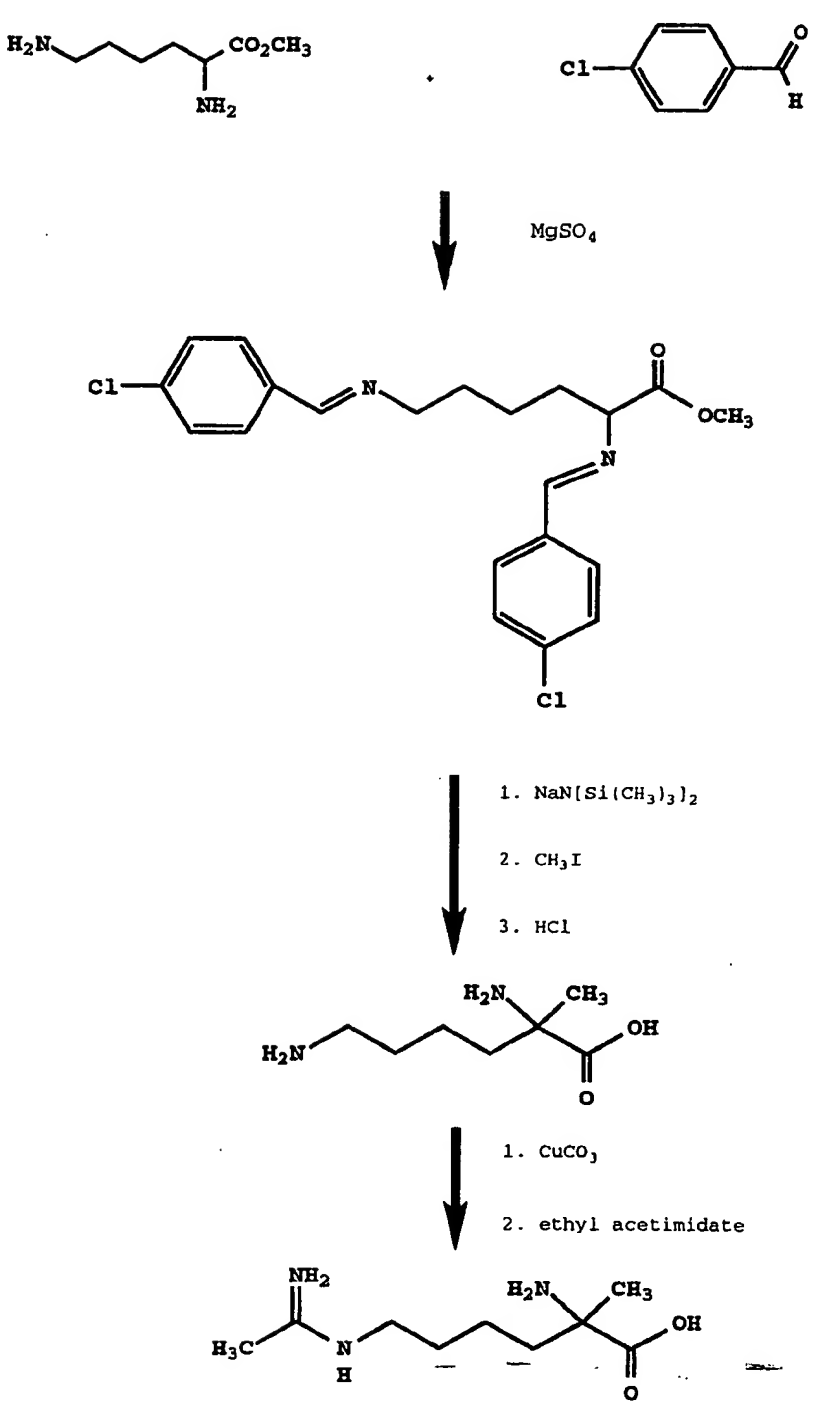
The term "prodrug" refers to a compound that is made more active *in vivo*.

As used herein, reference to "treatment" of a patient is intended to include prophylaxis.

All references, patents or applications, U.S. or foreign, cited in the application are hereby incorporated by reference as if written herein.

Disclosed is a general synthetic processes useful in the preparation of the compounds of the present invention.

SCHEME I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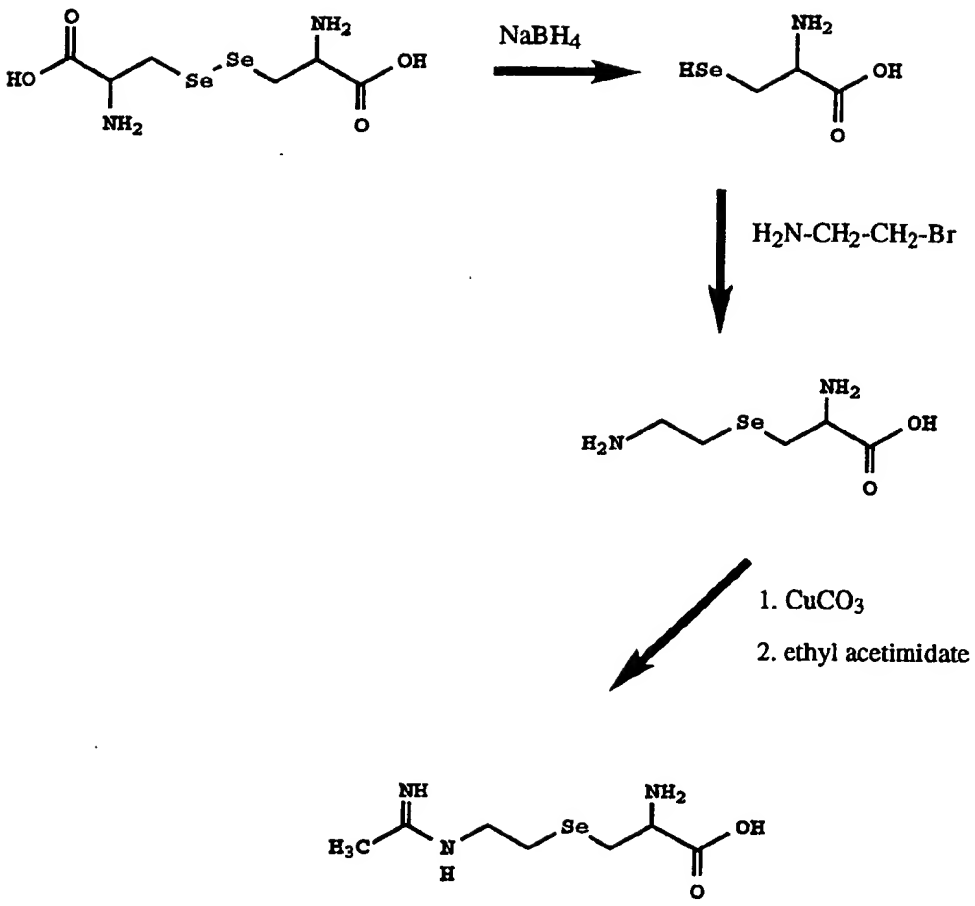

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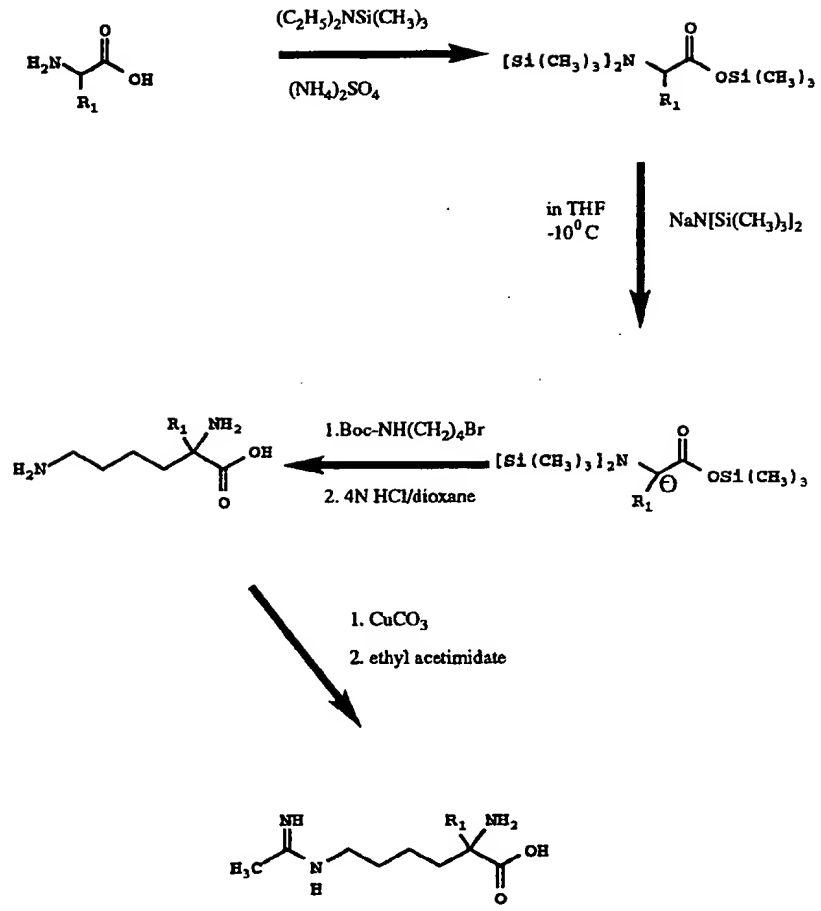
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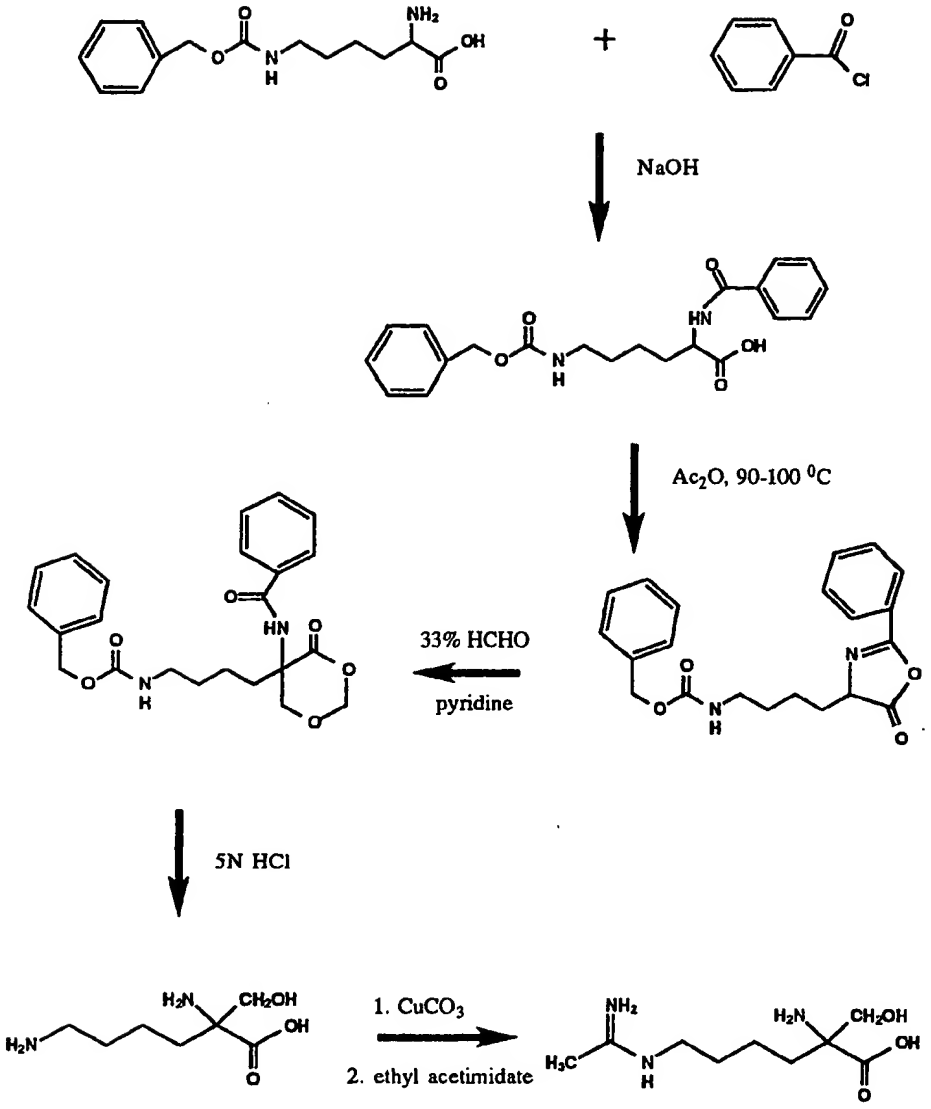
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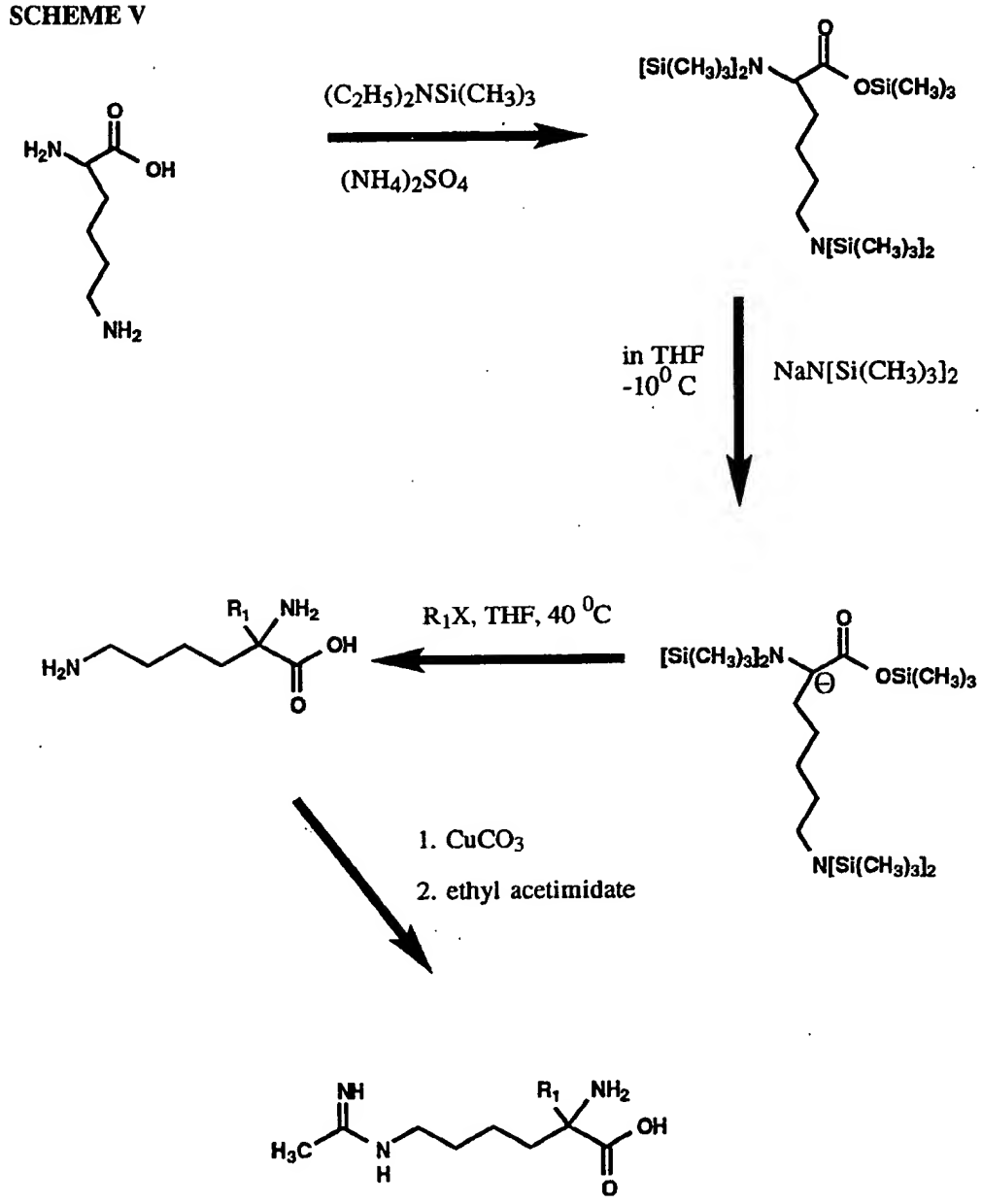
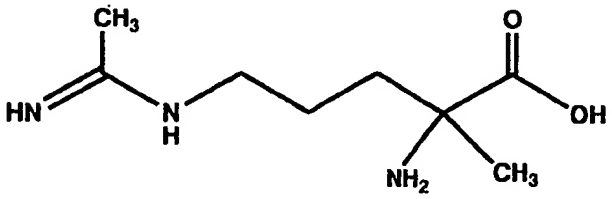
SCHEME V


$(C_2H_5)_2NSi(CH_3)_3$
$(NH_4)_2SO_4$
$[Si(CH_3)_3]_2N$
$OSi(CH_3)_3$
$N[Si(CH_3)_3]_2$
in THF
-10^0 C
$NaN[Si(CH_3)_3]_2$
R_1X, THF, 40 ^{0}C
1. $CuCO_3$
2. ethyl acetimidate

The invention is further illustrated by the following examples:

EXAMPLE 1

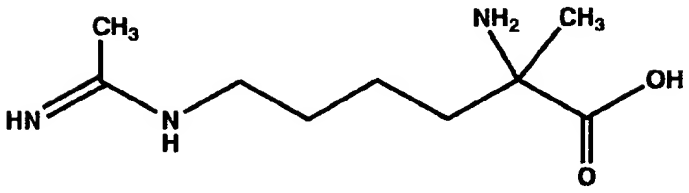
α-Methyl-Nδ-iminoethyl-ornithine



α-Methyl-D,L-ornithine hydrochloride (250 mg; 1.37 mmoles purchased from Sigma) was dissolved in 5 ml of water and 0.5 g of cupric carbonate was added. The mixture was stirred at 60 ^{0}C for 1 hr and filtered. The pH of the filtrate was adjusted to pH 9.5 with 0.5 N NaOH solution. Ethyl acetimidate hydrochloride (178 mg; 1.45 mmoles) was added and the pH was maintained at 9.0-9.5 for one hour. The solution was then adjusted to a pH of 7.5 and kept at room temperature for 12 hours. The solution was acidified with 1 N HCl to pH 4 and applied to a Dowex 50 X 4 (hydrogen form). The column was washed with water and then with 10 % pyridine. α-Methyl-Nδ-iminoethyl-ornithine was eluted from the column with 1 N $NH^{4}OH$. The ninhydrin positive fractions were combined and lyophilized. The residue was dissolved in 0.5 N of aqueous hydrochloric acid and re-lyophilized. α-Methyl-Nδ-iminoethyl-ornithine hydrochloride appears as white solids. (MH^{+} = 188); 1H-NMR (D20): d 1.4 (s, 3H); 1.45-1.9 (m, 4 H); 2.15 (s, 3 H); 3.3 (t, 2 H).

EXAMPLE 2

α-Methyl-Nε-iminoethyl-lysine

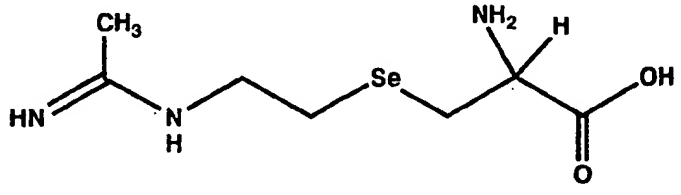


A suspension of lysine ethyl ester dihydrochloride (33 g; 0.14 mole) and $MgSO_4$ (34 g; 0.28 moles) in a solution of 4-chloro-benzaldehyde (39 g; 0.28 moles) and acetonitrile (500 ml) was stirred while N,N-diisopropylethylamine (36 g; 0.28 moles) was added in portions over 1/2 h. The mixture was stirred for 12 h, filtered, concentrated to a small volume, and diluted with 500 ml of ethyl ether. The ether solution was washed with 0.1% aqueous $NaHCO_3$, aqueous 2 N NaOH containing 2g/100 ml of $NH_2OH.HCl$, again with 0.1% aqueous $NaHCO_3$ and saturated aqueous NaCl. After drying with MgSO4 and removal of the solvent in vacuo, ethyl N, N'-di(4-chloro-phenylmethylene)-L-alanine was obtained as a clear liquid. The liquid was triturated with hexanes and the resulting solid was washed with hexanes for several times. This partially purified intermediate was dissolved in 200 ml of THF and stirred in an acetone/dry ice bath. Sodium bis-(trimethylsilyl)amide in THF (11 ml, 1 M solution) was added dropwisely over 30 min. After one hour, methyl iodide (0.8 g; 13 mmoles) in THF was added dropwisely. The reaction mixture was slowly warmed up to room temperature and stirred overnight. The mixture was diluted with water, and extracted with ethyl ether. The ether extract was washed with 0.1% aqueous NaHCO3 and saturated aqueous NaCl and concentrated to yield crude ethyl N.N'-di(4-chloro-phenylmethylene)-α-methyl-D,L-alanine (M + H= 434). This material (4 g) dissolved in

ethyl ether (100 ml) was stirred vigorously with 1 N HCl (50 ml) for 2 h, the layer was separated and the aqueous phase was washed with ethyl ether. The aqueous solution was further acidified by the addition of concentrated HCl to 6 N and was heated to reflux for 16 h. The solution was cooled to room temperature, and rotary evaporated to dryness. The residue was dissolved in water and applied to a Dowex 50 X 4 (hydrogen form).The column was washed with water, and then 10% pyridine. α-Methyl-D,L-lysine, (MH^+ H = 161) was eluted from the column with 1 M NH4OH. α-Methyl-D,L-lysine (300 mg) was dissolved in water and 0.5 g of cupric carbonate was added. The suspension was stirred at 600 C for 1 h and filtered. The filtrate was adjusted to pH 9.5 and ethyl acetimidate was added portionwise in 15 min. The pH was maintained at 9.0 to 9.5. After 1 h of stirring at room temperature, the solution was adjusted to pH 7 and the stirring was continued overnight. α-Methyl-Nε-iminoethyl-lysine was purified by Dowex 50X4 (hydrogen form) chromatography similarly described for α-Methyl-Nδ-iminoethyl-ornithine. MH^+ = 202; ^{1}H-NMR (D_2O): d 1.4 (s, 3H); 1.5-1.8 (m, 6 H); 2.05 (s, 3 H); 3.1 (t, 2 H).

EXAMPLE 3

Nε-iminoethyl-aminoethylselenocysteine

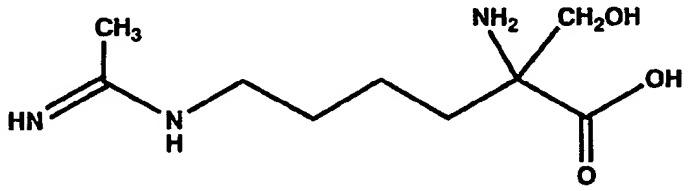


Seleno-D,L-cystine (117 mg; 0.5 mmoles, purchased from Sigma) was suspended in 15 ml of nitrogen gas-purged water. Sodium borohydride (38 mg; 1 mole) was added. The reaction mixture became clear in a few minutes. After 2 h

at room temperature, 2-bromoethylamine HCl (1.2 g; 6 mmoles) was added and the reaction mixture was stirred for 12 h. The reaction was applied on to a Dowex 50 X 4 (hydrogen form) column. The column was washed with water and 10% pyridine. 2-Aminoethyl-D,L-seleno-cysteine was eluted with 1 M NH4OH and, subsequently, treated with cupric carbonate and ethyl acetimidate as described for Nδ-iminoethyl-ornithine. Nε-iminoethyl-aminoethylselenocysteine was obtained as pale yellow solids. MH^+ = 254.

EXAMPLE 4

α-Hydroxymethyl-Nε-iminoethyl-lysine



To an ice-cold stirred mixture of Nε-Cbz-L-lysine (14 g; 0.05 moles, purchased from Sigma) in 2.5 N NaOH (24 ml), benzoyl chloride (10 g) was added gradually. The pH of the solution was maintained at 10.5-10.9 by addition of 2 N NaOH. The mixture was stirred at room temperature for 1 h and filtered. The filtrate was extracted with a small amount of ethyl acetate and the organic layer was dried over sodium sulfate. The solid was removed by filtration and the filtrate was evaporated to dryness. The remaining crude oily Nε-Cbz-Nα-benzoyl-lysine (6 g) was heated at 90-100^0 C with acetic anhydride (100 ml) for 30 min. The mixture was then evaporated. The residue was dissolved in pyridine and treated with aqueous formaldehyde (35% solution, Fisher). The mixture was stirred for 8 hr, then diluted. The reaction mixture was kept at 100C overnight the precipitated crude material was hydrolyzed in boiling

5 N HCl during 5 h. The reaction mixture was cooled and filtered before being evaporated. The solid residue was dissolved in water and passed through Dowex 50 X 4 (hydrogen form) column. α-Hydroxymethyl-D,L-lysine (MH^+ = 177) was eluted with 1 N NH_4OH and, subsequently, treated with cupric carbonate and ethyl acetimidate to form α-hydroxymethyl-Nε-iminoethyl-lysine similarly described for α-Methyl-Nδ-iminoethyl-ornithine. MH^+ = 218. ^1H-NMR (D_2O): d 1.1-1.8 (m, 6H); 2.1 (s, 3H); 3.1 (t, 2H); 3.6-3.9 (q(a,b), 2H).

The activity of the above listed compounds as NO synthase inhibitors have been determined in the following assays:

Citrulline Assay for Nitric Oxide Synthase

Nitric oxide synthase activity was measured by monitoring the conversion of [^3H]-arginine to [^3H]-citrulline. Mouse inducible nitric oxide synthase (miNOS) was prepared from an extract of LPS-treated RAW 264.7 cells and partially purified by DEAE-Sepharose chromatography. Rat brain constitutive nitric oxide synthase (rnNOS) was prepared from an extract of rat cerebellum and partially purified by DEAE-Sepharose chromatography. Enzyme and inhibitors were incubated at 37°C for 15 minutes in a reaction volume of 100 µL with the following components added to start the reaction: 50 mM Tris (pH 7.6), 1 mg/ml bovine serum albumin, 1 mM DTT, 2 mM $CaCl_2$, 10 µM FAD, 10 µM tetrahydrobiopterin, 30 µM L-arginine containing L-[2,3-^3H]-arginine at 300 cpm/pmole and 1 mM NADPH. For constitutive NOS, 50 nM calmodulin was also added. The reaction was terminated by addition of cold stop buffer containing 10 mM EGTA, 100 mM HEPES, pH 5.5 and 1 mM citrulline. [^3H]-Citrulline was separated by chromatography on Dowex 50W X-8 cation exchange resin and

radioactivity determined with a liquid scintillation counter.

Recombinant human inducible nitric oxide synthase (hiNOS) was extracted from Sf9 insect cells (infected with recombinant baculoviruses encoding a cDNA for human inducible nitric oxide synthase which has been isolated and purified from a lambda Zapll cDNA library made from RNA isolated from a colon sample from a patient with ulcerative colitis) and partially purified by DEAE-chromatography.

Raw Cell Nitrite Assay

RAW 264.7 cells are plated to confluency on a 96-well tissue culture plate grown overnight (17h) in the presence of LPS to induce NOS. A row of 3-6 wells were left untreated and served as controls for subtraction of nonspecific background. The media was removed from each well and the cells are washed twice with Krebs-Ringers-Hepes (25mM, pH 7.4) with 2 mg/ml glucose. The cells are then placed on ice and incubated with 50 µL of buffer containing L-arginine (30 µM) +/- inhibitors for 1h. The assay is initiated by warming the plate to 37°C in a water bath for 1h. Production of nitrite by intracellular iNOS is linear with time. To terminate the cellular assay, the plate of cells is placed on ice and the nitrite-containing buffer removed and analyzed for nitrite using a previously published fluorescent determination for nitrite. All values are the average of triplicate wells and are compared to a background-subtracted induced set of cells (100% value).

LPS is the abbreviation for lipopolysaccharide.

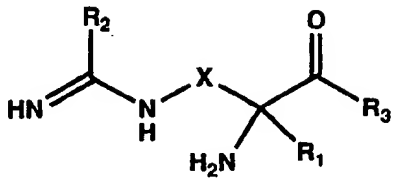
TABLE I

Compound	hiNOS	miNOS	rnNOS	Raw Cell
	IC_{50} [μM]			IC_{50} [μM]
Example 1	73.5	34.9	355.9	25.0
Example 2	26.6	7.2	192.8	52.5
Example 3	35.5	5.0	229.0	0.37
Example 4	39% Inh. @ 10 μM		8.6% Inh. @ 10 μM	

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

What is claimed is:

1. A Compound having the formula:



(I)

salts, pharmaceutically acceptable esters and prodrugs thereof, wherein

X is lower alkyl, lower alkenyl, lower alkynyl, $-(CH_2)_kQ(CH_2)_l-$ where k is 2 or 3, l is 1 or 2 and Q is O, Se, SiY_2 where Y is lower alkyl, $S(O)_z$ where z is 0, 1 or 2, NR where R is hydrogen or lower alkyl, or $-(CH_2)_mA(CH_2)_n-$ where m is 0, 1 or 2, n is 0, 1 or 2, A is a 3 to 6 membered aromatic hydrocarbon radical or 3 to 6 membered heterocyclic radical wherein 1 to 3 hetro atoms are oxygen, sulfur or nitrogen and wherein all said radicals may optionally be substituted by one or more substituents such as lower alkyl, lower alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino;

R_1 maybe a bond to X, or a radical selected from the group consisting of hydrogen (when Q is Se), hydroxyalkyl, lower alkyl,lower alkenyl, lower alkynyl, or haloalkyl and wherein all said radicals may optionally be substituted by one or more substituents such as lower alkyl, lower alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino;

R_2 is lower alkyl, lower alkenyl, lower alkynyl, aromatic hydrocarbon radical, haloalkyl, hydroxylamine or heterocyclic radical wherein 1 to 3 hetro atoms are

oxygen, sulfur or nitrogen and wherein all said radicals may optionally be substituted by one or more substituents such as lower alkyl, lower alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino; and

R_3 is amino, alkylamine, amino acid, hydroxy, lower alkoxy, tetrazole, or tetrazoloamine.

2. The compound as recited in claim 1 wherein

X is lower alkyl, lower alkenyl, lower alkynyl, $-(CH_2)_kQ(CH_2)_l-$ where k is 2 or 3, l is 1 or 2 and Q is O, Se, SiY_2 where Y is lower alkyl, $S(O)_z$ where z is 0, 1 or 2, NR where R is hydrogen or lower alkyl, or $-(CH_2)_mA(CH_2)_n-$ where m is 0, 1 or 2, n is 0, 1 or 2, A is a 3 to 6 membered aromatic hydrocarbon radical or heterocyclic radical wherein 1 to 3 hetro atoms are oxygen, sulfur or nitrogen and wherein all said radicals may optionally be substituted by one or more substituents such as lower alkyl, lower alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino;

R_1 maybe a bond to X, or a radical selected from the group consisting of hydrogen (when Q is Se), hydroxyalkyl, lower alkyl and haloalkyl;

R_2 is lower alkyl, lower alkenyl, lower alkynyl, aromatic hydrocarbon radical , haloalkyl, or hydroxylamine; and

R_3 is amino, alkylamine, natural amino acid, hydroxy, lower alkoxy, tetrazole, or tetrazoloamine.

3. The compound as recited in claim 1 wherein

X is a lower alkyl of 1 to about 6 carbon atoms, lower alkenyl of 1 to about 6 carbon atoms, lower alkynyl

of 1 to about 6 carbon atoms, $-(CH_2)_kQ(CH_2)_l-$ where k is 2 or 3, l is 1 or 2 and Q is O, Se, SiY_2 where Y is lower alkyl, S(O)z where z is 0, 1 or 2, or NR where R is H or lower alkyl; or $-(CH_2)_mA(CH_2)_n-$ where m is 0, 1 or 2, n is 0, 1 or 2, A is a 3 to 6 membered aromatic hydrocarbon radical or heterocyclic radical wherein 1 to 3 hetro atoms are oxygen, sulfur or nitrogen and wherein all said radicals may optionally be substituted by one or more substituents such as lower alkyl, lower alkoxy, hydroxy, halogen, nitro, cyano, trifluoroalkyl and amino;

R_1 is hydrogen (when Q is Se), a hydroxyalkyl group of 1 to about 4 carbon atoms, a lower alkyl group of 1 to about 4 carbon atoms or a haloalkyl group of 1 to about 4 carbon atoms;

R_2 is lower alkyl of 1 to about 4 carbon atoms, lower alkenyl of 1 to about 4 carbon atoms alkynyl of 1 to about 4 carbon atoms, aromatic hydrocarbon radical of 3 to about 6 carbon atoms and haloalkyl of 1 to about 4 carbon atoms; and

R_3 is an amino, alkylamine of 1 to about 4 carbon atoms, hydroxy, lower alkoxy of 1 to about 4 carbon atoms, tetrazole, tetrazoloamine, or natural amino acid.

4. The compound as recited in claim 1 wherein

X is an alkylene group having 3 to 5 carbon atoms and which may optionally be substituted by one or more C_{1-3} alkyl; a group of formula $-(CH_2)_kQ(CH_2)_l-$ where k is 2 or 3, l is 1 or 2 and Q is O, Se, S(O)z where z is 0, 1 or 2; or a group of formula $-(CH_2)_mA(CH_2)_n-$ where m is 0, 1 or 2, n is 0, 1 or 2, A is a 3 to 6 membered carbocyclic or heterocyclic ring;

R1 is hydrogen (when Q is Se), a hydroxyalkyl group of 1 to about 4 carbon atoms, a lower alkyl radical of 1 to about 4 carbon atoms;

R_2 is lower alkyl radical of 1 to about 4 carbon atoms; and

R_3 is an amino, alkylamine of 1 to about 4 carbon atoms, hydroxy, lower alkoxy group of 1 to about 4 carbon atoms.

5. The compound as recited in claim 1 selected from the group consisting of α-Methyl-Nδ-iminoethyl-ornithine, α-Methyl-Nε-iminoethyl-lysine, α-Methyl-Nε-iminoethyl-aminoethylselenocysteine and α-Hydroxymethyl-Nε-iminoethyl-lysine.

6. A pharmaceutical composition comprising a compound as recited in claim 1 together with a pharmaceutically acceptable carrier.

7. A pharmaceutical composition comprising a compound as recited in claim 2 together with a pharmaceutically acceptable carrier.

8. A pharmaceutical composition comprising a compound as recited in claim 3 together with a pharmaceutically acceptable carrier.

9. A pharmaceutical composition comprising a compound as recited in claim 4 together with a pharmaceutically acceptable carrier.

10. A pharmaceutical composition comprising a compound as recited in claim 5 together with a pharmaceutically acceptable carrier.

11. A method of inhibiting nitric oxide synthesis in a subject in need of such inhibition by administering a therapeutically effective amount of the compound as is recited in Claim 1.

12. A method of inhibiting nitric oxide synthesis in a subject in need of such inhibition by administering a therapeutically effective amount of the compound as is recited in Claim 2.

13. A method of inhibiting nitric oxide synthesis in a subject in need of such inhibition by administering a therapeutically effective amount of the compound as is recited in Claim 3.

14. A method of inhibiting nitric oxide synthesis in a subject in need of such inhibition by administering a therapeutically effective amount of the compound as is recited in Claim 4.

15. A method of inhibiting nitric oxide synthesis in a subject in need of such inhibition by administering a therapeutically effective amount of the compound as is recited in Claim 5.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/03589

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C257/14 C07C391/00 A61K31/195 A61K31/095

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 24126 (CORNELL RESEARCH FOUNDATION) 9 December 1993 cited in the application see page 3, line 8 - line 16; claims	1-5
A	WO,A,93 13055 (THE WELLCOME FOUNDATION) 8 July 1993 cited in the application see page 5, line 2 - line 8; claims see page 16, line 5 - line 25	1-5

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 June 1995	- 6. 07. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Seufert, G

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/03589

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9324126	09-12-93	US-A-	5281627	25-01-94
		EP-A-	0649303	26-04-95
WO-A-9313055	08-07-93	AU-B-	3169293	28-07-93
		EP-A-	0618898	12-10-94
		JP-T-	7502512	16-03-95